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12a. DISTRIBUTION / AVAILABILITY STATEMENT

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12 b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

During the past three years we have focused on three specific aims: (1) understanding the mechanism of ice-binding by antifreeze polypeptides (AFPs), (2) synthesis and characterization of peptides (CBPs) that alter the morphology of a mineral, calcite, and (3) characterizing the interaction between a specific CBP and calcite. In the course of pursuing aim (1), we discovered, in the longhorn sculpin, a new class (type IV) of antifreeze protein and have determined completely both its protein and DNA sequences. It contains 108 amino acids and, we believe, based on secondary structure analysis, folds into a 4-helix bundle.

We have designed and synthesized an α-helical peptide designed *de novo* to bind to the prism face of calcite. This peptide has a remarkable effect on calcite crystal morphology: at low temperatures, in its helical form, it does appear to bind to a prism face, but when the peptide is unfolded, it causes epitaxial growth off the rhombohedral surfaces of calcite seed crystals, resulting in very unusual morphology. *This is perhaps the first example of a rationally designed, morphology controlling mineral binding peptide* We have also synthesized a helical phosphopeptide which appears to bind to the basal face of calcite.

14. SUBJECT TERMS

Antifreeze protein, biomineralization, calcite, longhorn sculpin, protein structure, crystallization

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ANTIFREEZE POLYPEPTIDES AS BIOMINERALIZATION MODELS

FINAL PROGRESS REPORT

RICHARD A. LAURSEN

25 OCTOBER 1997

U.S. ARMY RESEARCH OFFICE

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I FORWARD

Not applicable

II. TABLE OF CONTENTS

Not applicable

III. LIST OF APPENDIXES

Listed in part VII below

IV. BODY OF REPORT

A. Statement of Problem Studied

Our specific aims as stated at the beginning of this award three years ago were (1) to test our previously formulated ice-binding hypotheses by studying other type I antifreeze polypeptides (AFPs), e.g., from sculpin; (2) to design an optimal helix template in order to (3) synthesize a calcite-binding peptide (CBP); (4) to study the effect of the CBP on calcite crystal morphology; and (5) to study the effects of growing calcite crystals in gels. We have made significant progress towards the first four aims, and in addition have made an interesting discovery of a new class of AFP, as described more fully in publications and manuscripts (see **part C** below and **Appendix**).

B. Summary of Most Important Results

1. Discovery and characterization of a new type of antifreeze protein. While attempting to repeat the isolation of a previously described type I AFP from sculpin, we happened to discover in the longhorn sculpin, which reportedly contained no AFP, the first example (called LS-12) of an entirely new class of AFP. We have purified LS-12, determined its entire amino acid sequence, as well as the DNA sequence encoding the precursor protein. LS-12 contains 108 amino acids and, based on chemical, spectroscopic and computational analysis, probably folds to form a four-helix bundle with a hydrophobic core and a polar surface, which contains many of the amino acids (Thr, Gln, Ser) typically associated with ice-binding. The significance of this new AFP is that the ice-binding face probably is comprised of two helixes and thus may have a larger, flat area than seen in other types of AFP. Thus helix bundle structures, which are rather rigid, could be useful templates for constructing other crystal binding proteins.

Ice crystals formed in the presence of LS-12 grow as hexagonal right trapezohedra, rather than hexagonal bipyramids seen with type I AFPs. Analysis of the geometry of these crystals suggest that LS-12 is binding to the $\{3\bar{1}\bar{2}1\}$ adsorption plane, though we have not yet formulated a detailed protein-ice binding model.

2. Structure-Function studies on type I AFPs. To better understand the requirements for ice-binding in the α-helical type I AFPs, we made a series of analogs of the winter flounder AFP in which the four Thr residues thought to be involved in ice binding were systematically replaced by Ser, the side chain of which has more rotational mobility than Thr on a helix surface. We found that only the two central Thr residues (at positions 13 and 24) were critical for antifreeze activity, suggesting that the binding domains may be limited to the central portion of the helix. We have also synthesized a 45-residue AFP (SS-8) from shorthorn sculpin, in order to demonstrate that we can make a peptide this large, and obtained a product that has good activity.

We plan to do structure-function studies using analogs of SS-8 to better define the ice-binding motifs for this AFP, which are currently unknown.

3. Control of calcite crystal morphology by a peptide designed to bind to the prism face. We have synthesized an α-helical peptide (CBP1) designed to bind to the $\{1\bar{1}0\}$ prism faces of calcite, and found that it alters the morphology of growing calcite crystals in a rather striking way (Figure 8). When added to growing rhombohedral seed crystals, CBP1 can have two very distinct effects: at 3°C, where CBP1 is 89% helical, the crystals assume a prismatic habit; at 25°C, where CBP1 is largely unstructured, studded crystals result from epitaxial growth off each of the six rhombohedral surfaces. Furthermore, when the peptide is removed from the growth medium, the crystals regrow to express the more stable rhombohedral faces. We believe that the epitaxial type growth results from non-specific binding of a polyanion, since other acidic peptides and even poly(methylmethacrylate) cause similar growth. Much of our recent effort has been to try to characterize the surfaces of these new crystals, using a variety of techniques: x-ray powder diffraction, scanning electron microscopy, atomic force microscopy, confocal microscopy, etc.--not all of which have been very helpful, the problem being that the crystals are very small and the newly expressed surfaces are not smooth. Nevertheless, we have demonstrated, we believe for the first time, that a polypeptide of defined structure can alter the morphology of a mineral in a remarkable way, resulting in several forms, depending on growth conditions.

4. Helical phosphopeptides appear to bind to the basal faces of calcite. We have synthesized a series of helical peptides, modeled after AFPs, in which one or more Thr residues are substituted by phosphoserines. These phosphopeptides cause calcite crystals to assume a rounded shape, which can most easily be explained by binding to the basal plane of calcite. Molecular modeling suggests a reason for this. Unlike a carboxylate group (e.g., on the side chain of Asp), which is planar and can replace a carbonate in calcite by lying in the same plane, the phosphate group is tetrahedral and can best replace a carbonate by binding perpendicular to to the calcium ion layer

5. Relationship to ARO Mission

The objective of this project has been to gain fundamental information about the mode of interaction of polypeptides with crystalline inorganic surfaces, and to learn how to control crystal size, shape and deposition. As such, this work cuts across several areas of interest to the ARO: structure-activity relationships of macromolecules and protein interactions (Biosciences); the chemistry of interfaces and surfaces (Chemistry); and the synthesis and processing of materials, such as composites (Materials Sciences). Bone and Shell are examples of biological composite materials which have great strength due to protein-mineral interactions.

C. Publications and Technical Reports

1. Publications and manuscripts

a. G. Deng, D. W. Andrews and R.A. Laursen, Amino Acid Sequence of a New Type of Antifreeze Protein, from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis, FEBS Letters* **402,** 17-20 (1997).

b. D. B. DeOliveira and R.A. Laursen, Control of Calcite Crystal Morphology by a Peptide Designed to Bind to a Specific Surface, *J. Am. Chem. Soc.* (in press).

c. Z. Zhao, G. Deng, Q. Lui and R.A. Laursen, Cloning and Sequencing of cDNA Encoding the LS-12 Antifreeze Protein from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis* (submitted).

d. G. Deng and R.A. Laursen, Isolation and Characterization of a New Type of Antifreeze Protein from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis* (in preparation).

e. W. Zhang and R.A. Laursen, Structure-Function Relationships in an Antifreeze Polypeptide: The Effect of Hydroxyamino Acid Rigidity on Activity (in preparation).

f. G. Deng, Isolation and Characterization of an Antifreeze Protein from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis,* Ph.D. Thesis, Boston University, 1997.

2. Presentations

a R. Laursen, "Coping with the Cold: Fish Antifreeze Polypeptides," Lectures presented at the U.S. Coast Guard Academy, Groton, CT (10/24/95); Department of Chemistry, Clark University, Worcester, MA (10/30/95); Department of Chemistry, Colby College, Waterville, ME (10/30/95); Department of Chemistry, Eastern Nazerene College, Quincy, MA (3/18/96); Department of Chemistry, Brown University, Providence, RI (4/9/96).

b. R.A. Laursen, D. Wen, G. Deng and D. DeOliviera, "Binding of α-Helical Peptides to Crystal Surfaces," Fifth Antifreeze Protein Workshop, Toronto, ON, October 20-21, 1995.

c. G. Deng, D. Andrews and R.A. Laursen, "Structure of a New Type of Antifreeze Protein, from the Longhorn Sculpin, *Myoxocdphalus octodecimspinosis*", 10th Symposium of the Protein Society, San Jose, CA, August 3-7, 1996.

d. R.A. Laursen, D.W. Andrews and G. Deng, "Structure of a New Type of Antifreeze Protein, from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis,*" XIth International Conference on Methods in Protein Structure Analysis, Annecy, France, September 1-5, 1996.

e. R.A. Laursen, "Antifreeze Polypeptides: Models for Understanding Biomineralization and the Control of Crystal Morphology," C.N.R.S., Gif-sur-Yvette, France (9/6/96).

f. W. Zhang and R.A. Laursen, "Effects of Serine Substitution on Activity of an Antifreeze Polypeptide," 11th Symposium of the Protein Society, Boston, MA, July 12-16, 1997.

g. D. DeOliveira and R.A. Laursen, "Control of Calcite Crystal Morphology by a Designed Calcite Binding Peptide," 11th Symposium of the Protein Society, Boston, MA, July 12-16, 1997.

h. Z. Lian and R. A. Laursen, "Synthetic Phosphopeptides which Bind to the (001) Basal Face of Calcite," 11th Symposium of the Protein Society, Boston, MA, July 12-16, 1997.

D. Participating Scientific Personnel

Richard A. Laursen (P.I)
Gejing Deng (Ph.D. awarded May 1997)
Daniel DeOliveira (Ph.D. expected May 1998)
Wei Zhang (Ph.D. Candidate)
Zhirui Lian (Ph.D. Candidate)

V. REPORT OF INVENTIONS

An invention disclosure describing calcite crystal morphology modification by peptides of defined sequence and by polyanions will be filed shortly with the Boston University Patent Office.

VI. BIBLIOGRAPHY

Not applicable

VII. APPENDIXES (attached)

1. G. Deng, D. W. Andrews and R.A. Laursen, Amino Acid Sequence of a New Type of Antifreeze Protein, from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis, FEBS Letters* **402,** 17-20 (1997). **[Published article]**

2. D. B. DeOliveira and R.A. Laursen, Control of Calcite Crystal Morphology by a Peptide Designed to Bind to a Specific Surface, *J. Am. Chem. Soc.* (in press). **[Galley proof]**

3. Z. Zhao, G. Deng, Q. Lui and R.A. Laursen, Cloning and Sequencing of cDNA Encoding the LS-12 Antifreeze Protein from the Longhorn Sculpin, *Myoxocephalus octodecimspinosis* (submitted). **[Manuscript of submitted article]**